14) A New Technique for Detecting Erythrocyte Membrane Characteristics in SHRSP. Yukio Yamori, Kazuko Mori, Yasuo Nara and Akira Ooshima. Department of Pathology, Shimane Medical University, Izumo 693, Japan.

INTRODUCTION Previously we reported that erythrocyte membrane from SHR and stroke-prone SHR (SHRSP) showed characteristic features in sodium ion permeability, osmotic fragility detected by the technique of a coil-planet-centrifugation, microviscosity detected by a microviscosimeter and also in the permeability of lipophilic ions. Reports from other laboratories also indicated that net-sodium flux or lithium-sodium counter transport or simply sodium content is different in red cells from SHR or patients with essential hypertension. These findings in SHR and in humans with essential hypertension suggested that biomembrane abnormalities of erythrocytes may be related to genetic factors of the pathogenesis of genetic hypertension. Since there is the possibility that such an abnormality related to genetic factors is detectable before the development of hypertension, erythrocytes may be utilized for the early detection of genetic disposition, i.e., the prescience of hypertension which is expected to contribute to the prevention of hypertensive diseases.

In the present study we applied our new method for observing hemolysis caused by clemastine to the differentiation of erythrocytes from SHR, SHRSP and their age-matched normotensive Wistar-Kyoto rats (WKY). Clemastine is a tertiary amine like chlorpromazine. These tertiary amines are known to hemolyze red blood cells in a higher concentration, although the detailed mechanism of hemolysis has not been clarified.

MATERIALS AND METHODS Male SHRSP, stroke-resistant SHR (SHRSR) and WKY at the age of 6 weeks and 3 months, 6 of each group, were anesthetized to obtain blood samples into heparinized syringes by cardiac puncture. The blood was centrifuged at 1500 g for 10 min at 4°C to separate the red blood cells. These cells were then washed 3 times with isotonic NaCl phosphate buffer and finally adjusted to 40 ± 1 % in hematocrit value. 0.2 ml of erythrocyte suspension was mixed with 2 ml of isotonic NaCl phosphate buffer containing different concentrations of clemastine hydrogen fumarate (3×10^{-4} , 4×10^{-4} and 5×10^{-4} M), incubated at 37°C and taken out at 10-min intervals. Non-hemolyzed erythrocytes were separated by centrifugation at 1500 g for 3 min and the supernatant was assayed for hemoglobin, spectrophotometrically at 543 nm wave length.

RESULTS AND DISCUSSION Erythrocytes from any strain of rats were not hemolyzed at the concentration of 3×10^{-4} M, and 50 % were hemolyzed at 5×10^{-4} M during the incubation of 20 min. At the concentration of 4×10^{-4} M hemolysis was increased with the incubation time as follows; about 5, 35 and 70 % at 20, 40 and 60 min of incubation, respectively. Therefore, the optimum concentration for this experiment was proven to be 4×10^{-4} M.

In 3 month-old rats, hemolysis of the erythrocytes from WKY, SHRSR and SHRSP at 40 min of incubation was 55.2 ± 4.6 , 40.9 ± 1.0 and 54.4 ± 1.5 %, respectively. There were significant differences between SHRSR and WKY or between SHRSR and SHRSP. Hemolysis at 50 min of incubation in WKY, SHRSR and SHRSP was 85.1 ± 3.5 , 73.4 ± 0.9 and 87.7 ± 1.0 %, respectively. Hemolysis at 60 min of incubation in WKY and SHRSP was nearly 100 %, 97.4 ± 1.9 and 97.9 ± 1.1 %, respectively. However, hemolysis in SHRSR was 90.2 ± 0.5 %. Though hemolysis was increased in accordance with the lapse of time, significant differences between SHRSR and WKY, and between SHRSR and SHRSP remained.

In 6 week-old rats, erythrocytes were more fragile than those in 3 month-old rats so that hemolysis in the former group was greater by 10 to 20 % than that in the latter.

This method is simple enough to determine the characteristics of erythrocyte membrane within a short period and may hopefully contribute to the differentiation of erythrocytes from rats with genetic disposition to severe hypertension or to stroke.

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